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Full Paper

Trimetazidine prevents diabetic cardiomyopathy by inhibiting Nox2/TRPC3-induced oxidative stress

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ABSTRACT

Diabetic cardiomyopathy (DCM) is characterized by cardiac hypertrophy, fibrosis, oxidative stress and inflammation. Trimetazidine (TMZ), a potent metabolism modulator, has been shown to be cardioprotective in experimental models of ischaemia-reperfusion and type 2 diabetes-induced cardiomyopathy. The present study examined whether TMZ inhibits cardiomyopathy induced by insulin-dependent type 1 diabetes. Wistar rats were randomly divided into control group (vehicle alone), diabetes mellitus (DM; induced by streptozocin (STZ) injection) group and DM treated with TMZ (DM/TMZ) group. Cardiac function, histology, plasma biochemistry and molecular mechanism were assessed. STZ induced diabetes in rats as indicated by hyperglycemia, increased and decreased levels of advanced glycation end products (AGEs) and insulin respectively. Diabetic rats were characterized by left ventricular dysfunction, cardiachypertrophy and fibrosis and signs of inflammation and oxidative stress in the myocardium, which were accompanied by elevated levels of NADPH oxidase 2 (Nox2) and transient receptor potential channel 3 (TRPC3) in the heart. TMZ treatment ameliorated diabetes-associated structural and functional alterations by inhibiting Nox2 and TRPC3 without having any effects on glucose, insulin and AGEs levels. These results suggest that TMZ could be used as a therapy to treat cardiomyopathy associated with type 1 induced diabetes mellitus.

1. Introduction

Diabetes mellitus (DM)-induced diabetic cardiomyopathy (DCM), as initially described in 1972 by Rubler,[1] is characterized by myocardial dysfunction as well as cardiac remodeling that is unrelated to coronary artery heart disease and/or hypertension. DCM is a major cause of death in DM patients, with a 2–5 fold higher risk than in non-diabetic patients suffering from cardiomyopathy.[2] It is well established that DCM is attributed to multiple factors, including hyperglycemia, oxidative stress and inflammation.[3] Among them, oxidative stress plays a crucial role. NADPH oxidase 2 (Nox2) induces oxidative stress by virtue of it's catalytic activity in diabetic hearts that ultimately leads to fibrosis.[4] Transient receptor potential channel 3 (TRPC3), together with Nox2, play critical roles in cardiac remodeling.[5] At present, there is no effective therapeutic strategy for DCM and it's management is a significant challenge. Consequently, there is an urgent need to develop novel, more efficient therapeutic approaches for clinical management of this disease.

Trimetazidine (TMZ), a partial fatty-acid-oxidation inhibitor, has been emerging as a promising therapeutic option for DCM and it is available for use in more than 80 countries.[6] The beneficial effects of TMZ on the cardiomyopathy including ischemic and non-ischemic heart failure has been reported.[7–9] It has been recently suggested that TMZ could be used to prevent and treat DCM[10] and shown in type 1 or type 2 diabetic rat models that TMZ improves cardiac function and prevents cardiac fibrosis in DCM by modifying

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membrane homeostasis and counteracting metabolic disorder.[11,12] However, the underlying mechanism of beneficial effects afforded by TMZ in context of DCM is yet unknown. We have hypothesized that TMZ could prevent increase in Nox2 and TRPC3 in diabetic hearts.

Therefore, in the present study, we investigated the effect of TMZ on STZ-induced DCM and the underlying mechanism of this effect. We have found that TMZ attenuated DCM by inhibiting inflammation and oxidative stress. This is the first report ever to demonstrate that TMZ inhibits reactive oxygen species (ROS) production by inhibiting Nox2/TRPC3, which, in turn, prevents cardiac dysfunction in type 1 diabetes.

2. Materials and methods

2.1. Animal models and study design

The study conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and reviewed and approved by Institutional Animal Care and Use Committee of Sun Yat-sen University. Experiments were performed on eight-weeks old male Wistar rats (180–210 g) provided by the Animal Centre of Sun Yat-Sen University. All the animal were raised with a 12-h light/dark cycle and fed with a standard chow diet *ad libitum* (n = 10/group). To induce diabetes mellitus, rats were injected with a single dose of streptozotocin (STZ, S0130, Sigma–Aldrich, 55 mg/kg; i.p.) prepared in 0.1 M sodium citrate buffer (pH = 4.5). A separate group of rats were injected with citrate buffer only (control group). Five days after STZ injection, the fasting blood glucose level was measured using Accu-CHEK active blood glucose meter (Roche, Mannheim, Germany). Rats with blood glucose levels >11.1 mmol/L were considered as diabetic, and then randomly divided into two groups: untreated group (DM) and TMZ-treated diabetic group (DM/TMZ). One week after DM was induced, DM/TMZ rats were treated with TMZ (Fengyaotonghui Chemical Products Co., Ltd., China, 10 mg/kg/day) or vehicle for 11 weeks.

2.2. Hemodynamic assessment

Twelve weeks after STZ injection, cardiac function was determined by hemodynamic analysis. Parameters measured included the maximal rate of LV pressure rise (dP/dt_{max}, in mmHg/s) and LV end-systolic pressure (LVESP) to reflect LV contraction, the minimal rate of LV pressure fall (dP/dt_{min}, in mmHg/s) and LV end-diastolic pressure (LVEDP) as measurements of relaxation. The parameters were assessed with SPR-838 pressure catheter system (Millar, ADInstruments Inc., Houston, TX, USA) after rats were anesthetized (pentobarbital, 50–60 mg/kg, i.p.) as described previously.[13] Briefly, following rat ventilation, the pressure transducer catheter was inserted into the right carotid artery and directed to the left ventricle. To calculate hemodynamic parameters, as teady-state period of at least 30 seconds was selected. The other LV functional parameters, including heart rate (HR), mean arterial pressure (MAP), cardiac output (CO) and isovolumic relaxation time (Tau), were recorded by a data acquisition system (LabChart, ADInstruments Inc., CO, USA).

After these measurements were performed, rats were sacrificed, hearts and blood were collected for experiments described below.

2.3. Tissue and plasma collection for biochemical analysis

Hearts were harvested and washed with chilled PBS to clear out blood. Heart weight (HW) was normalized to tibial length (TL) and the ratios of HW to TL were calculated. The blood glucose levels were measured in venous blood, and plasma was prepared for further analysis. The inflammation markers, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and C-reactive protein (CRP) in plasma and myocardial tissue, as well as insulin and AGEs levels in plasma, were measured by commercially available kits (RAT TNF-α, IL-6, CRP and INSULIN ELISA kits, JissKang Biotech, Qingdao, China; RAT Advanced Glycation End Products ELISA kits, SenBeiJia Biological, Nanjing, China) according to manufacturer's instructions.

2.4. Histology

The hearts were harvested after animals were sacrificed, and then cut into 0.2 cm-thick sections. Some sections were rapidly fixed in 4% paraformaldehyde for histological analysis. The fixed sections were then paraffin embedded for haematoxylin and eosin (H&E) or Sirius Red staining. H&E staining was used to determine the cardiomyocyte cross-sectional areas. Slides were scanned by an Axisplus image-capturing system (Zeiss, Germany) and the cell sizes were quantified by Image-Pro Plus (Media Cybernetics, Bethesda, MD, USA). Cross-sectional areas were randomly selected with a magnification of 400 ×. At least five fields and 50 cells per animal were calculated for analysis of cardiac hypertrophy. To determine the tissue fibrosis, cardiac sections were stained with Sirius Red (Huamaike Biotech, Beijing, China). Images were taken at a final magnification of 200 × and analyzed by Image-Pro Plus. The level of tissue fibrosis was calculated by the ratio of fibrotic area compared to the total analyzed area (expressed as the percentage).

2.5. Immunohistochemical staining

The cardiac 8-hydroxy-2' -deoxyguanosine (8-OHdG) and CD68 were visualized by immunohistochemical analysis. After dewaxing, sections of each heart were hydrated with graded series of ethanol, followed by treating with 0.3% H_2O_2 for 30 minutes. After washing with Tris buffered solution, the sections were blocked with 5% goat serum. The sections were incubated with mouse monoclonal antibody against 8-OHdG (Santa Cruz#sc-393871, 1:100) or CD68 (Santa Cruz#sc-20060, 1:100) and a biotinylated goat anti-mouse IgG (VectorLaboratories, CA,USA)at 4 °C. After that, the sections were incubated with biotinylated anti-mouse immunogloblin for 30 min and, then, incubated with horseradish peroxidase-conjugated streptavidin for 30 min. The peroxidase binding sites and nuclei were visualized with diaminobenzidine (DAB, Sigma–Aldrich, USA) and hematoxylin respectively. The density of positive staining (IOD) was analyzed using Image-Pro Plus.

2.6. Measurements of total anti-oxidative capacity (T-AOC), superoxide dismutase (SOD) activity and malondialdehyde (MDA) levels

Some of the 0.2 cm-thick sections were frozen in liquid nitrogen and then stored at −80 °C for ELISA, biochemical and molecular biological analysis using commercial kits according to manufacturer's instructions. Protein was extracted from cardiac tissues (Nanjing Jiancheng Chemical Co, Nanjing, China) and the concentrations were calculated with BCA Protein Assay Kit (Pierce, Rockford, IL, USA). Total Antioxidant Capacity (T-AOC) and SOD activity from tissue were determined by T-AOC (according to the redox potential of Fe3+-TPTZ) and SOD activity (WST-1 method) detection kits (Nanjing Jiancheng Chemical Co, Nanjing, China), respectively. The enzyme activities were expressed as U/mg protein. The MDA level, an indicator of lipid peroxidation, was measured by TBARS assay kit (Beyotime, China). The result was expressed as nmol/g tissue.

2.7. Real-time quantitative PCR for gene expression analysis

Total RNAs were extracted from LV tissue using 1 ml Trizol (Generay, Shanghai, China) per 100 mg of tissue. The quality of total RNAs was confirmed by gel electrophoresis. For reverse transcription, the samples were treated with gDNA wiper MIX and then transcribed into cDNA using HiScript II 1st Strand cDNA Synthesis Kit (Vazyme, Nanjing, China). The amplification was performed on QuantStudio 3 Real-Time PCR System (Applied Biosystems, Foster City, CA) with 2 × SYBR Green PCR Premix HS Taq (Genview, Beijing, China). The relative gene expressions were calculated using the ΔCt (sample Ct minus β-actin Ct) and ΔΔCt (sample delta Ct minus comparator delta Ct) methods as previously described.[14]

2.8. Western blot analysis

Cardiac tissues were lysed in cold RIPA lysis buffer (AmsBio) and protease inhibitor cocktail (P8340, Sigma) was added to extract protein according to the manufacturer's instructions. Protein concentration was determined by BCA method (Pierce). Equal amounts of proteins (30–50 μg) were separated by SDS-PAGE, then proteins were transferred to PVDF membrane (Millipore). After blocked by 5% (w/v) nonfat milk in TBST buffer at room temperature for 1 hr, proteins were probed with primary antibodies, anti-gp91-phox (Santa cruz #sc-130543, 1:500), anti-TRPC3 (Santa cruz #sc-514670. 1:500) and anti-β-actin (CST #3700, 1:1000), at 4 °C for overnight. The membrane was then incubated with goat-anti-mouse HRP conjugated IgG (Jackson, 1:3000) at room temperature for 45 min. The target proteins were detected by ECL chemiluminescence kit (Pierce) and analyzed by ImageLab software (Bio-Rad).

2.9. Statistical analysis

All data are expressed as mean ± standard deviation (SD). All the analyses were performed using the statistical program GraphPad (version 6.0). One-way ANOVA following post hoc Tukey's t-test were used to compare values among groups. A value of $p < 0.05$ was regarded as significant.

3. Results

3.1. Trimetazidine did not affect development of diabetes in streptozocin-treated rats

The body weight, blood glucose levels, as well plasma AGEs and insulin levels were measured in control, DM and DM/TMZ rats (Table 1). At 12 weeks-time point, DM mice were significantly lighter than controls (Table 1). In contrast, there was no statistical significant difference between DM/TMZ mice and control mice (Table 1). After 12 weeks follow up, DM and DM/TMZ rats were still hyperglycemic and had higher and lower AGEs and insulin plasma levels respectively (Table 1). DM rats also demonstrated a trend of increased total cholesterol (T-CHO) and triglyceride (TG) content in plasma when compared to control rats, but this difference was not statistically significant. In summary, TMZ treatment did not have any significant effect on the body weight, hyperglycemia, AGEs and plasma insulin levels oftype 1 diabetic rats.

3.2. Trimetazidine counteracted diabetes-induced cardiac dysfunction

LV diastolic and systolic functions were evaluated by measuring LVEDP, LVESP and calculating dP/dt_{max} and dP/dt_{min} (Table 2). Diabetes-induced LV diastolic dysfunction was observed in diabetic rats as indicated by reduced dP/dt_{min} and elevated LVEDP 12 weeks after STZ injection. Diabetic rats also exhibited significantly reduced dP/dt_{max} and LVESP, showing a long-term diabetes-induced decline in systolic function. Rats treated by TMZ were protected from diabetes-induced LV dysfunction, as demonstrated by improved dP/dt_{max} and decreased LVEDP. TMZ treatment showed a trend to enhance LVESP ($p = 0.075$) anddP/dt_{min} ($p = 0.118$). In addition, MAP, HR and CO were significantly decreased while Tau was increased ($p = 0.073$) in diabetic rats. TMZ significantly improved MAP, and had a tendency to enhance CO ($p = 0.062$) and reduce Tau ($p = 0.075$). However, diabetes or TMZ treatment showed no effects on HR.

3.3. Trimetazidine protected against diabetes-induced LV hypertrophy and fibrosis

STZ-induced diabetes was associated with hypertrophy of cardiomyocytes as reflected by an increase in cross-sectional area of these cells (Fig. 1A,B) and heart-to-tibial length ratio (HW/TL) (Fig. 1C), both of which were prevented by TMZ. Markers of LV hypertrophy including atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) were significantly elevated in DM rats (Fig. 1D,E), which was attenuated by TMZ.

LV collagen content was increased at the study endpoint in DM rats as revealed by the Sirius Red staining (Fig. 2A). Administration of TMZ prevented this diabetes-induced increase in LV collagen content (Fig. 2B,C). The expression of TGF-β1 and Smad-3 were also significantly elevated in the myocardium of diabetic rats (Fig. 2D,E). TMZ inhibited increase in Smad-3, but not TGF-β1expression (Fig. 2D,E).

3.4. Trimetazidine inhibited oxidative stress induced by hyperglycemia

Hyperglycemia-induced oxidative stress plays a critical role in the pathogenesis of DCM. The T-AOC in cardiac tissues in DCM group at 12 weeks-time point decreased significantly when

Table 1
Metabolic and physiological parameters of control, DM and DM/TMZ groups. Values are mean ± SD; n = 10; *, **, ***: $p < 0.05$, $p < 0.01$ and $p < 0.001$ compared to control rats.

	Control	DM	DM/TMZ
Rats Number	6	8	8
Body weight (0 week) (g)	222.33 ± 5.69	232.36 ± 9.04	228.70 ± 10.17
Body weight (12 weeks) (g)	355.17 ± 29.61	316.00 ± 15.10 *	323.70 ± 39.70
Blood glucose (mmol/L)	7.95 ± 0.76	15.15 ± 5.38 *	13.51 ± 5.05
Plasma AGE (μg/mL)	56.92 ± 37.04	88.75 ± 41.65 *	88.99 ± 33.56 *
Plasma insulin (mU/L)	18.37 ± 2.32	14.47 ± 1.28 ***	14.98 ± 1.24 **
Total cholesterol (mmol/L)	1.73 ± 0.33	2.07 ± 0.44	2.29 ± 0.36
Triglyceride (mmol/L)	0.43 ± 0.16	0.58 ± 0.23	0.66 ± 0.23

Table 2
The cardiac function of control, DM and DM/TMZ groups. Values are mean ± SD; n = 10; *, **, ***: $p < 0.05$, $p < 0.01$ and $p < 0.001$ compared to control rats. #, ##: $p < 0.05$ and $p < 0.01$ compared to DM rats.

	Control	DM	DM/TMZ
LVEDP (mmHg)	2.06 ± 0.76	5.85 ± 1.92 *	3.22 ± 1.61 #
LVESP (mmHg)	130.34 ± 7.76	105.58 ± 15.38 *	125.86 ± 20.38
dP/dt_{min} (mmHg/s)	−12550 ± 1866	−8504 ± 2088 *	−10068 ± 1422 *
dP/dt_{max} (mmHg/s)	11140 ± 546	8084 ± 2067*	10672 ± 1986 #
MAP (mmHg)	123.11 ± 4.71	97.17 ± 15.12*	119.53 ± 13.77 ##
CO (μL/min)	71725 ± 47860	32135 ± 17577 **	44522 ± 23808
Tau (ms)	7.25 ± 0.81	12.39 ± 7.70	9.31 ± 2.36
HR (bpm)	431.07 ± 5.32	397.83 ± 18.72 *	391.55 ± 43.36

compared to the controls (Fig. 3A). Treatment with TMZ counteracted this decrease (Fig. 3A). Similar trends were observed with SOD-2 activity, although significance was not reached (Fig. 3B). MDA myocardial content was elevated in DM rats, which was inhibited by TMZ (Fig. 3C).In the myocardium of DM rats, 8OHdG positive-staining and Nox2, p22phox and p47phox mRNA levels were significantly increased when compared to controls (Fig. 3D–H), while SOD-2 mRNA level was significantly decreased (Fig. 3I). Except p22phox, all of these diabetes-induced markers of oxidative stress were significantly attenuated by TMZ.

3.5. Trimetazidine inhibited increase of Nox2 and TRPC3 in DM rats

It has been demonstrated that Nox2 and TRPC3 play critical roles in promoting ROS production under different conditions. Western blotting demonstrated significant increases of myocardial Nox2 and TRPC3 in DM group (Fig. 4), which were inhibited by TMZ.

3.6. Trimetazidine attenuated inflammation induced by oxidative stress

Tumor necrosis factor (TNF-α) and interleukin-6 (IL-6) plasma levels were significantly increased DM group at 12 weeks-time point (Fig. 5A). However, the level of these two factors were not significantly different between DM and control rats in the myocardium (Fig. 5B). TMZ reduced levels of TNF-α and IL-6 in plasma and cardiac tissue (Fig. 5A,B). Interestingly, levels of C-reactive protein (CRP) was not significantly different between experimental groups (Fig. 5A,B). CD68, the marker of macrophage infiltration, was significantly elevated in the heart of diabetic rats (Fig. 5C,D), which was inhibited by TMZ. The expression of the proinflammatory markers in the myocardium, including IL-1β, monocyte chemoattractant protein-1 (MCP-1), vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule-1

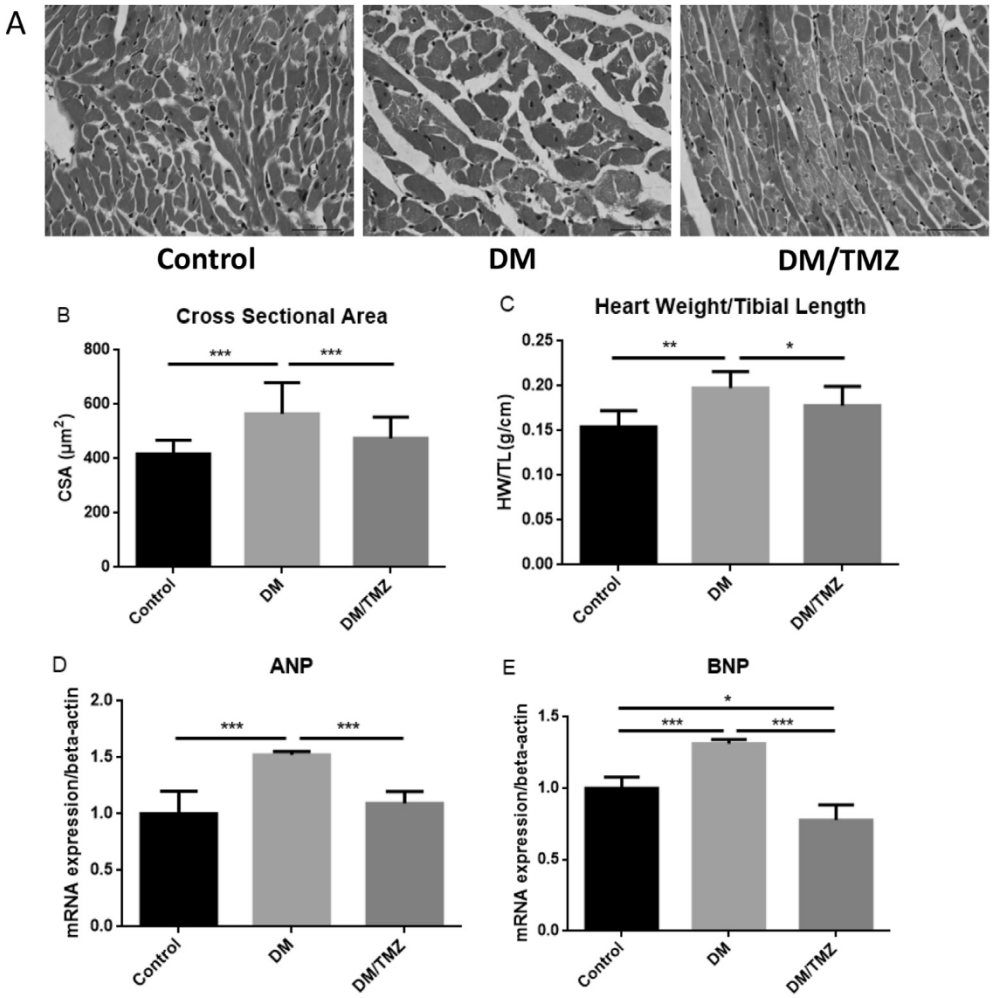

Fig. 1. TMZ treatment prevented diabetes-induced cardiac hypertrophy. (A), H&E staining of cardiac tissues with the magnification of 400 × . The scale bar in A represents 50 microns, n = 6–8. Diabetic hearts showed significant increase in cross sectional area (B), heart weight-to-tibial length (C), as well as hypertrophic biomarkers ANP (D) and BNP (E), n = 4 in D to E. TMZ decreased size of cardiomyocytes and inhibited the expression of hypertrophic biomarkers (B–E). Values are mean ± SD; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

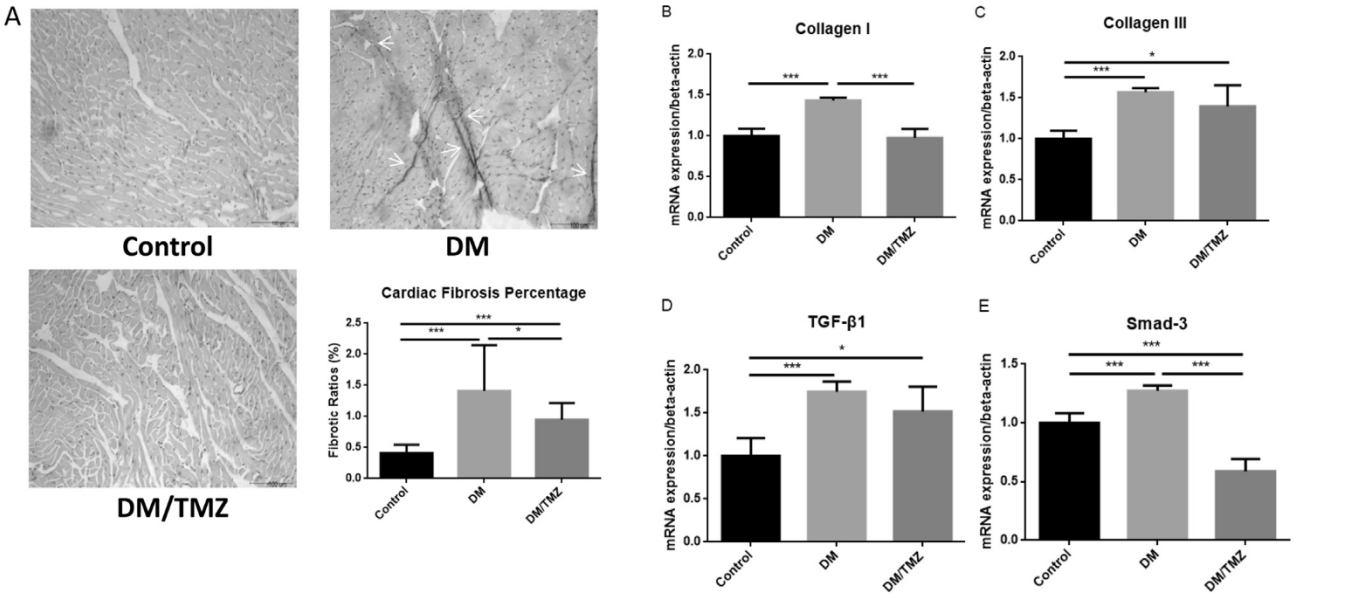

Fig. 2. TMZ prevented diabetes-induced cardiac fibrosis. (A) Sirius red staining is indicating collagen deposition and implying the extent of cardiac fibrosis with the magnification of 200 × . The arrowhead indicated the collagen deposition. The scale bar in A represents 100 microns, n = 6–8. (B–E). The fibrosis-related genes expression of TGF-β1, Smad-3, collagen I and collagen III was significantly elevated by diabetes as reflected by increased mRNAs, which was prevented by TMZ, except in cases of TGF-β1 and collagen III, n = 4–6 in B to E. Values are mean ± SD; *, $p < 0.05$; ***, $p < 0.001$.

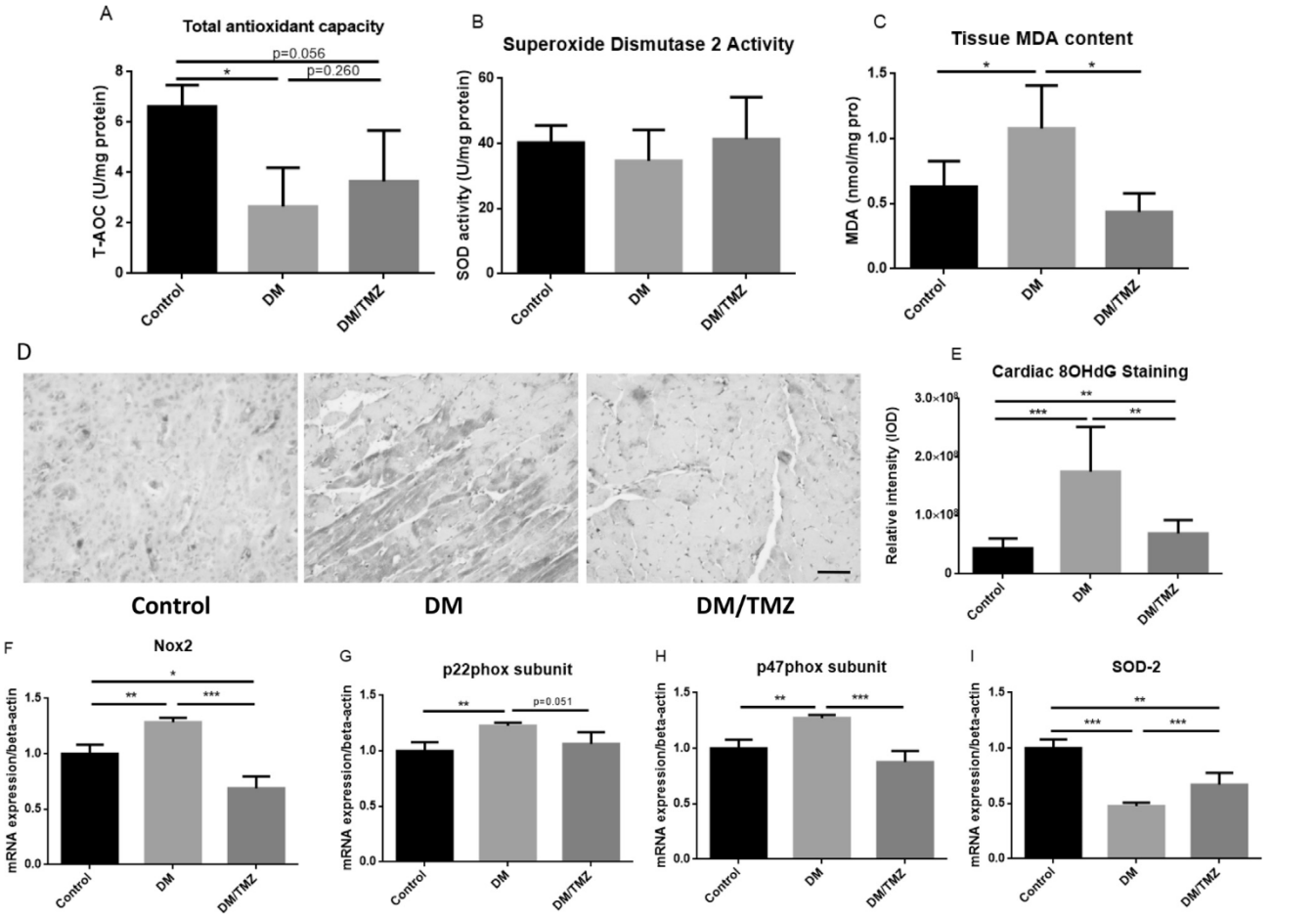

Fig. 3. TMZ prevented oxidative stress in diabetic myocardium. Oxidative stress in the myocardial tissues was determined by measuring (A) total anti-oxidative stress capacity (T-AOC), (B) SOD activity and (C) MDA concentration. (D–E) The 8OHdG positive staining sites of cardiac tissues with the magnification of 200 × , indicated oxidative stress in DM rats. The scale bar in D represents 100 microns, n = 6–8 in A to E.(F–I) NADPH oxidase subunits or SOD-2 mRNA expression detected by reverse transcriptase-polymerase chain reaction: Nox2, p22phox, p47phox mRNAs were increased and SOD-2mRNA was decreased in diabetic hearts. TMZ counteracted all diabetes-induced alterations, n = 4 in F to I. Values are mean ± SD; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

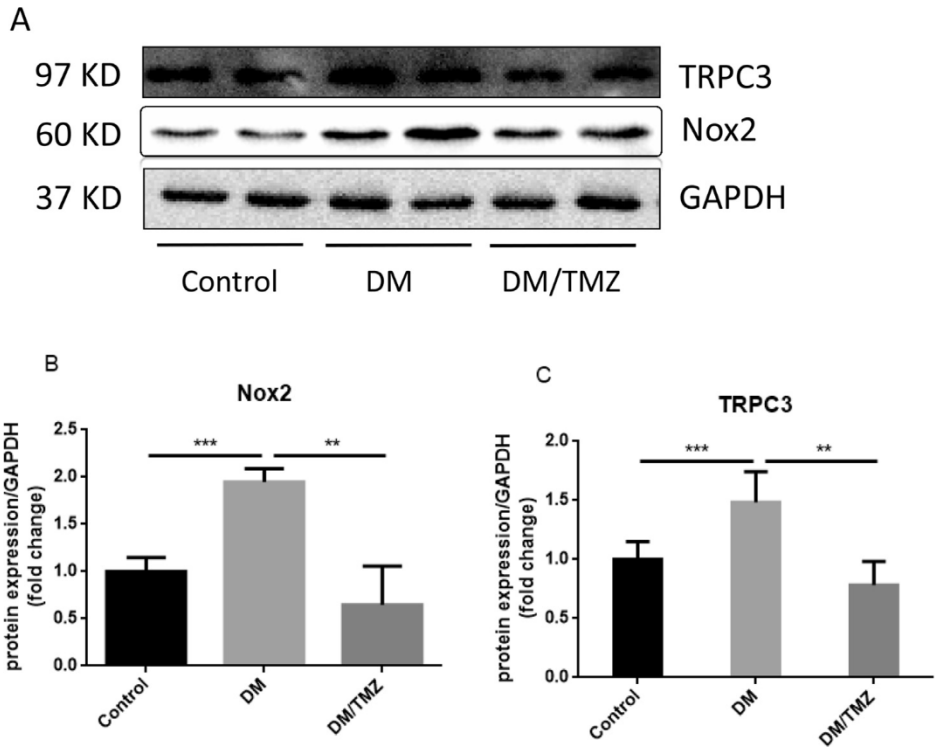

Fig. 4. TMZ inhibited Nox2 and TRPC3 expression. (A) Western blot to detect the Nox2 as well as TRPC3 expression in cardiac tissues. (B) and (C) Graphs corresponding to A, n = 4. Values are mean ± SD; **, $p < 0.01$; ***, $p < 0.001$.

(ICAM-1), were significantly increased in DM group, which was inhibited by TMZ (Fig. 5E–H).

4. Discussion

In the present study, we developed a type 1 diabetes-induced cardiomyopathy characterized by cardiac hypertrophy, fibrosis, oxidative stress, and inflammation. TMZ treatment significantly reduced oxidative stress and inflammation, and prevented hypertrophy and fibrosis. This suggests that TMZ protects against diabetic cardiomyopathy.

Previous studies proposed use TMZ as a therapeutic strategy to treat DCM.[10] This notion has been validated in different experimental models.[11,12,15] However, the underlying mechanism of TMZ-mediated beneficial effects remained unclear. Here, we demonstrated that TMZ prevents oxidative stress induced by elevated Nox2 and TRPC3 suggesting that this is probably the mechanism underlying TMZ cardioprotective effect.

Diabetes is a complex metabolic disease characterized by hyperglycemia and gross metabolic alterations that lead to many complications closely associated with inflammation and oxidative stress.[16] It is generally accepted that ROS formation provokes inflammatory responses.[17,18] Therefore, inhibition of ROS production could be an effective method for treating DCM. Some studies demonstrated that Nox2 and TRPC3 stimulate ROS production, which, in turn, alter cardiac structure and function[4,19] often leading to heart failure and, even death.[20] At present, there are no therapeutics specifically tailored for DCM and, consequently, developing pharmacological agents for management of this condition is warranted. TMZ, a well-established drug present for many years in clinical practice, has been shown to be useful in treatment of myocardial infarction (MI) and heart failure (HF).[7,8]Repurposing already marketed drugs for other indications is a highly efficient drug development strategy[21] and developing TMZ as a therapeutic tool for DCM would be an effective strategy to treat this condition.

In the present study, TMZ treatment counteracted diabetes-induced changes in Nox2, p22phox, SOD-2 and p47phox mRNAs and LV superoxide levels. The proteins of Nox2, as well as TRPC3, an another key factor in intracellular ROS formation,[5] were significantly increased in hearts in diabetic rats, which was inhibited by TMZ. It has been shown that inhibition of Nox2 by TMZ reverses myocardial dysfunction.[22] Although Nox2 interacts withTRPC3 during ROS production,[23] there was no research examining the impact of TMZ on TRPC3. Here, we demonstrated that TMZ inhibit both TRPC3 and Nox2, indicating that anti-oxidative stress properties of TMZ may be due to preventing ROS generation.

Many studies have reported an increase in cardiac tissue and/or blood concentrations of inflammatory cytokines in various rodent models of diabetes, suggesting an essential role of inflammation in development of DCM.[24] In line with this notion, our study showed that inflammatory response occurred in DM rats as reflected by increased levels of TNF-α and IL-6 in the heart and blood, and macrophage infiltration in the myocardium. These findings are in agreement with clinical trials reporting higher levels of pro-inflammatory factors in DM patients.[25] Following treatment with TMZ, myocardial and plasma levels of TNF-α and IL-6 in DM rats dramatically declined along with the decreasing macrophage infiltration and IL-1β, MCP-1, ICAM-1 and VCAM-1 expression in myocardial tissue. This is in agreement with a recent study showing that administration of TMZ decreases pro-inflammatory cytokines level in cardiac tissues in pressure overload models.[26] The relationship between CRP and diabetes is still controversial. Many studies have suggested that CRP is involved in inflammatory events during diabetes especially type 2.[27,28] Our results did not show any differences in plasma or cardiac tissue CRP level between experimental groups, which is consistent with a notion that CRP is not associated with DM.[29]

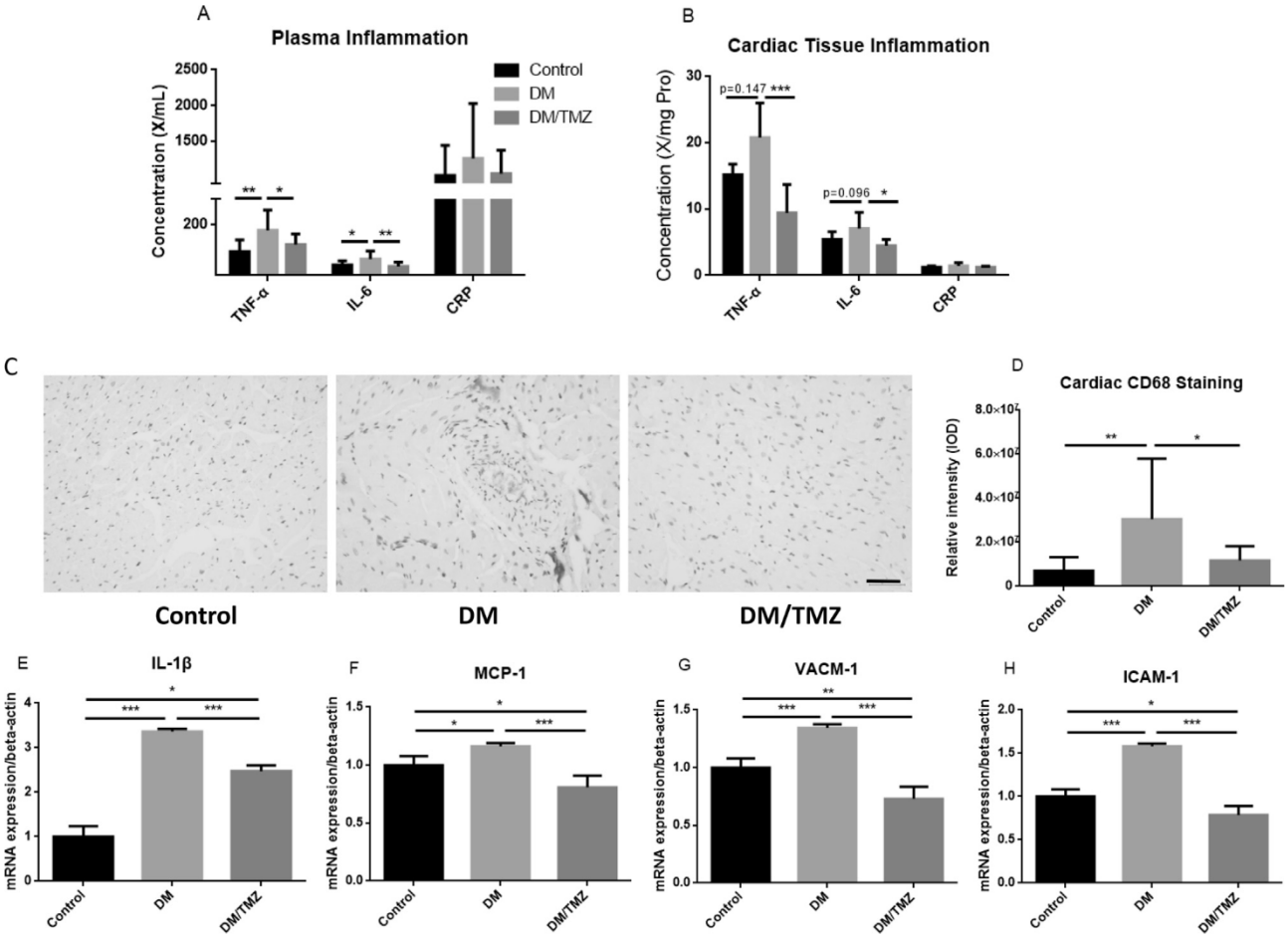

Fig. 5. TMZ mitigated diabetes-induced inflammation response. The concentration of TNF-α, IL-6 and CRP of plasma or myocardial tissue were detected by ELISA (A–B). TMZ mitigated diabetes-induced inflammation. "X" refers to nmol, nmol and μmol for TNF-α, IL-6 and CRP respectively. (C–D) CD68 positive staining sites of cardiac tissues. The magnification in C is 200 × and the scale bar represents 100 microns. n = 6–8 in A to D. (E–H) The inflammatory signal-related genes mRNA levels of IL-1β, MCP-1, VACM-1, and ICAM-1 were significantly increased by diabetes, which was inhibited by TMZ treatment. n = 4 in E to H. Values are mean ± SD; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

The present study demonstrated that TMZ significantly attenuated symptoms of DCM by inhibiting oxidative stress and inflammation. Twelve weeks following STZ injection, LV systolic dysfunction became statistically significant. These functional alterations were inhibited or substantially attenuated in rats treated with TMZ. In DM experimental group, we have also found an increase in expression of LV hypertrophy markers (ANP and BNP) and an increase in size of cardiomyocytes, which is in agreement with previous findings in STZ-induced diabetes.[30] In accord to previous reports,[31] we have also observed an increase in LV collagen content and TGF-β1 expression in DM group. Taken all together, DM hearts had all crucial features of diabetic cardiomyopathy. TMZ treatment attenuated interstitial collagen content and altered gene expression with few exceptions, i.e. the transcription of TGF-β1 and collagen III was not significantly affected by TMZ. In a previous study, it has been reported that TMZ treatment decreased high total cholesterol (T-CHO) and triglyceride (TG) concentration of type 2 diabetic rats.[11] In the present study, we showed that type 1 diabetes had a trend to increase T-CHO and TG levels, but TMZ did not have any effect on those levels. These differences could be due to many factors, including due to different experimental models studied.

In summary, our results suggest that oxidative stress and inflammation play critical roles in the pathophysiology of DCM. TMZ treatment prevents development of cardiac dysfunction in DCM by preventing hypertrophy and fibrosis through decreasing oxidative stress and inhibiting inflammatory response. An inhibition of oxidative stress was associated with decreases in Nox2 and TRPC3. Based on our findings, we believe that TMZ deserves to be seriously considered as a potential therapeutic strategy against DCM. Further studies are required to fully elucidate molecular mechanism of TMZ-mediated effects.

5. Conclusion

The present study demonstrated that TMZ prevents development of DCM in diabetes type 1, despite persistent hyperglycemia and insulin deficiency. The beneficial effect of TMZ seems to be associated with Nox2/TRPC inhibition and consequent anti-oxidative and anti-inflammatory effects that lead to inhibition of myocardial hypertrophy and fibrosis. These findings suggest that TMZ could be used to treat patients suffering from DCM.

Conflict of interest

The authors declare that there is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jphs.2019.01.016.

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